

On-Glass Integration of Thin Film Devices for Monitoring of Cell Bioluminescence



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Abstract This paper reports the development of a miniaturized lab-on-glass, suitable for the on-chip detection of living cell bioluminescence and their on-chip thermal treatments. The glass substrate hosts, on one side, hydrogenated amorphous silicon diodes, working as both temperature sensors and photosensors, and, on the other side, transparent thin films acting as heating sources. The main challenge of the work is the determination of the correct fabrication recipes in order to satisfy the compatibility of different microelectronic steps. The measured uniformity of temperature distribution, sensitivity of the temperature sensors, reverse dark current and spectral response of the photosensors demonstrate the successful technological integration and the suitability of the developed lab-on-glass to control the cell temperature and detect the BL emission with high sensitivity.

Keywords Lab-on-chip · Amorphous silicon · Bioluminescence · Cells
Indium tin oxide · Photosensor · Thin film heater

1 Introduction

The development of cost-effective, on-site analytical methods is an urgent need for diagnostics, monitoring food safety and detecting environmental pollution. Many laboratory procedures can be simplified through lab-on-chip (LoC) systems

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exploiting microfabricated sensors and heaters with microfluidics [1]. Moreover, to obtain highly valuable information, especially related to bioactivity and toxicology, living cells can be used as sensing elements and integrated in LoC. Indeed whole-cell biosensors have shown the potential to complement both laboratory-based and on-field analytical methods for the detection of general stress conditions, cyto- and genotoxic compounds, organic xenobiotics and metals [2–4].

Additionally, bioluminescence (BL), thanks to its peculiar features can be easily implemented in LoC as detection technique [5, 6]. BL reactions are characterized by high quantum yield emission and low background which results in high detectability and sensitivity of BL-based assays. In addition, BL does not require an external excitation light source and therefore it is an ideal detection principle for miniaturized and low weight systems.

The recent availability of new BL probes that emit at different wavelengths, such as new reporter genes or luciferase mutants, could also enable multiplexing detection relying on the spatial and spectral resolution of BL signals [7].

In this framework, we integrate on a single glass substrate different thin film technologies [8] in order to develop a lab-on-chip system suitable for on-chip thermal treatments of living cells and simultaneous on-chip detection [9] of their bioluminescence emission [10]. The main challenge of the work is the determination of the correct fabrication recipes of several electronic devices requiring different technological specifications.

2 System Structure and Operation

The developed system integrates on a single glass substrate different thin film technologies in order to obtain a multifunctional platform suitable for on-chip thermal treatments and on-chip monitoring of cell bioluminescence.

In particular, a $5 \times 5 \text{ cm}^2$ glass substrate includes:

- transparent thin film resistors acting as heating sources, in order to provide the required temperature to the cells;
- hydrogenated amorphous silicon (a-Si:H) diodes, acting as temperature sensors, to monitor the temperature distribution on the active area of the heater;
- a-Si:H diodes, acting as photosensors, to detect the bioluminescence provided by the analytes during the biomolecular recognition.

Figure 1 depicts the integrated thin film devices and their combination with the biological sample, which is contained in a Polydimethylsiloxane (PDMS) microfluidic network. The presented structure reduces the distance between the detector and the bioluminescence source, minimizing the optical losses and system dimensions.

In order to perform the thermal treatment of the cells, an Indium Tin Oxide (ITO) layer is driven by a custom-made electronics, applying a voltage that increases the sample temperature by conduction heat transfer. The temperature is stabilized at the set-point (37°C) using a software Proportional-Integral-Derivative algorithm, which

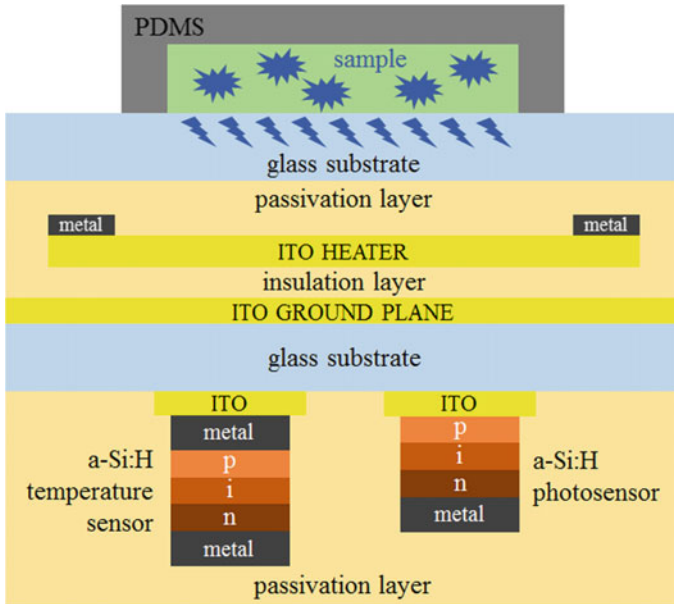


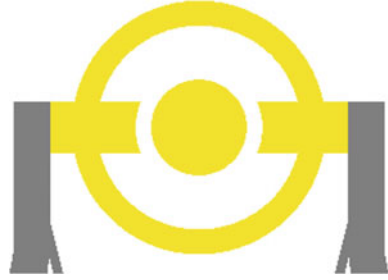
Fig. 1 Cross section of the LoC: the bottom glass substrate hosts the transparent heater on the top side and the a-Si:H sensors on the bottom side, while the top substrate hosts the PDMS microfluidic network

takes, as input, the temperature inferred by a-Si:H diode [11]. This is possible since the voltage across the diode varies linearly with the temperature when it is biased with a constant current. In order to correctly infer the temperature, a metal layer covering the ITO contact of the temperature sensors is used to shield the light radiations (see Fig. 1).

During the thermal control, the detection of the BL signal emitted by the cells is performed by the amorphous silicon photosensors, thanks to the transparency of the thin film heater that is crucial for the correct transmission of the emitted light.

The preliminary characterization of a first prototype highlighted a crosstalk between the heater and the a-Si:H sensors. In particular, it has been found that the biasing of the heater affects the current of the thin film sensors, because the leakage current through the glass resulting from the voltage applied to the heaters interferes with the current flowing through the sensors. A ITO transparent ground plane has then been inserted between the thin film heaters and the glass to ensure an electrical path of this current to ground. Furthermore, an insulation layer has been deposited on the ground plane before the heater fabrication.

Fig. 2 Top view of the heater geometry. The yellow region represents the ITO active layer, while the grey regions are the metal electrical contacts



2.1 Heater and a-Si:H Sensor Design

Geometry and thickness of the heater have been optimized, by using COMSOL Multiphysics, to satisfy requirements of temperature uniformity of treated active area, cell spatial displacement and transparency to light. Basing on the simulation results, we found that the geometry reported in Fig. 2 satisfies the biological specifications because:

1. the round shape allows for an optimal cell spatial distribution;
2. the temperature distribution has a standard deviation equal to ± 0.3 °C.

The material of the active area (yellow region in Fig. 2) is a 250 nm-thick ITO, which has a transmittance around 90% at wavelengths above 400 nm. Each heater has a circular shape with an active area of 0.18 cm^2 . The electrical pads (gray regions in Fig. 2) are a 30/150/30 nm-thick Cr/Al/Cr stacked structure.

The a-Si:H diodes are p-i-n stacked layers, whose thickness and energy gap have been designed [12] to match the photosensor responsivity with the BL emission spectrum, which is about 460 nm, and minimizing the reverse dark current.

3 Fabrication and Characterization

Heaters and thin film sensors have been fabricated using standard thin film micro-electronic technologies, and a careful tuning of sequence and parameters of the technological steps has been carried out to achieve the compatibility of the different processes. In particular, the a-Si:H diodes have been deposited by Plasma Enhanced Chemical Vapor Deposition in a three-chamber high vacuum system (GSI, Denver, CO, USA). The ITO film has been instead deposited by magnetron sputtering (MRC, Orangebourg, New York, NY, USA).

The fabrication steps of the whole structure are the following:

- Heater layer:
 1. deposition by magnetron sputtering of a 20 nm-thick ITO layer, which acts as transparent ground plane for the heaters;

2. deposition by spin coating of a 5 μm -thick SU-8 3005 (from MicroChem, MA, USA) insulation layer;
 3. deposition by magnetron sputtering of a 250 nm-thick ITO layer and its patterning by photolithography and dry etching processes for the fabrication of the heater central part;
 4. vacuum evaporation of 30/150/30 nm-thick Cr/Al/Cr stacked layer acting as electrical pads of the heaters and its patterning by photolithography and wet etching processes;
 5. deposition by spin coating of a 5 μm -thick SU-8 3005 passivation layer;
- Sensors layer:
 1. deposition by magnetron sputtering of a 100 nm-thick ITO layer, which acts as transparent bottom contact of the diodes;
 2. patterning of the ITO layer by photolithography and wet etching processes;
 3. deposition by Plasma Enhanced Chemical Vapor Deposition (PECVD) of the a-Si:H 10/150/30 nm-thick p-type/intrinsic/n-type stacked structure;
 4. deposition by vacuum evaporation of a 50 nm-thick chromium layer, which acts as top contact of the sensors;
 5. wet etching of the chromium and dry etching of the a-Si:H layers for the mesa patterning of the diodes;
 6. deposition by spin coating of a 5 μm -thick SU-8 3005 (from MicroChem, MA, USA) passivation layer and its patterning for opening via holes over the diodes;
 7. deposition by magnetron sputtering of a 150 nm-thick titanium/tungsten alloy layer and its patterning for the definition of the top contacts and of the connection to the pad contacts;
 8. deposition by spin coating of a 5 μm -thick SU-8 3005 passivation layer.

Figure 3 reports a picture of the fabricated chip.

The heaters, characterized using a thermo-camera FLIR A325 and a custom made electronic board, show a very good temperature uniformity according to the numerical analysis (Fig. 4). The a-Si:H photosensors show dark current below 10^{-10} A/cm² (which leads to a dark current noise of few fA) and responsivity around 200 mA/W in the BL emission spectrum range. The temperature sensors present a sensitivity of 3.2 mV/°C when biased at a forward constant current of 8 $\mu\text{A}/\text{cm}^2$. The performances of the a-Si:H sensors are comparable with those achievable with the state-of-the-art crystalline silicon sensors.

From these results, we infer that the integrated devices fulfill the requirements for a wide range of cell-based BL assays.

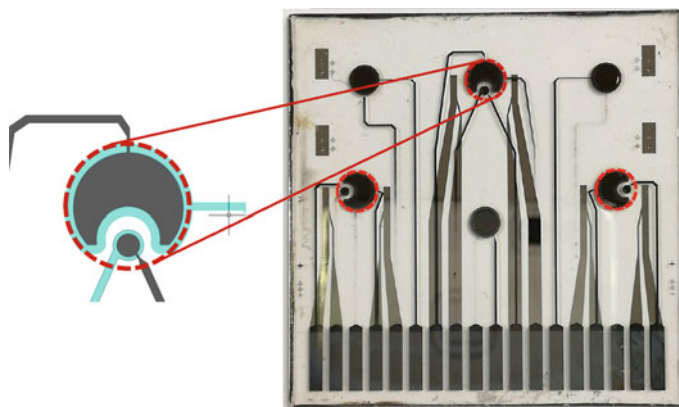


Fig. 3 Picture of the fabricated LoC, hosting heaters on the top glass side and sensors on the bottom side. The expanded area highlights the bottom glass moon-like shaped photosensors and circle-shaped temperature sensors

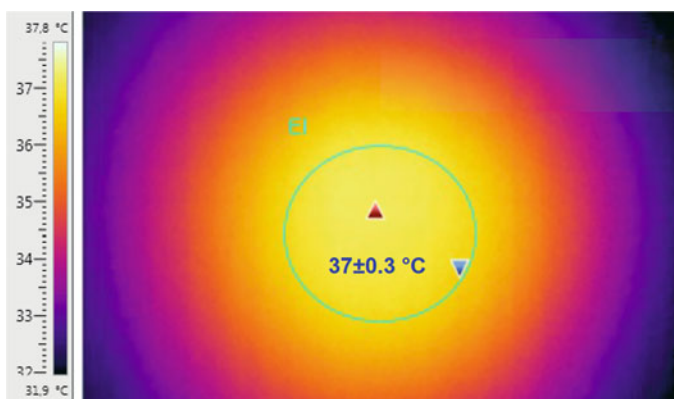


Fig. 4 Temperature distribution measured using a FLIR A325 thermocamera. The thermal energy is provided by the thin film heater driven by a custom electronic board. The green circle specifies the area of the photosensor aligned with the heater

4 Conclusions

In this work, an integrated lab-on-chip system designed to monitor and control the activity of living cells has been presented. The developed system-on-glass is based on thin film microelectronic technologies and integrates, on the same glass substrate, thin film heaters and amorphous silicon sensors in order to achieve a compact system to control the cell temperature and detect the BL emission with high sensitivity.

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